JOURNAL OF CLINICAL MICROBIOLOGY, May 1982, p. 860–864
0095-1137/82/050860-05$02.00/0

Vol. 15, No. 5

Comparison of Recovery of Organisms from Blood Cultures Diluted 10% (Volume/Volume) and 20% (Volume/Volume)

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Received 17 September 1981/Accepted 7 January 1982

We compared blood cultures that were diluted 1:5 (20%, vol/vol) and 1:10 (10%, vol/vol) and contained specimens from patients with suspected septicemia. *Streptococcus pneumoniae* was recovered significantly more frequently from blood cultures diluted 20%, whereas gram-negative bacilli, group D streptococci, *Staphylococcus aureus*, and *Candida* spp. were recovered significantly sooner and more frequently from blood cultures diluted 10%. Statistically significant differences in isolation rates, however, represented only a small number of patients for whom the positive cultures affected therapy. We conclude that as long as at least two separate sets of blood cultures are obtained per septic episode from each patient, a 1:5 to 1:10 blood/vented (aerobic) medium ratio provides acceptable results. Nevertheless, the results also demonstrate that blood cultures diluted 10% provided greater and faster yields than those provided by blood cultures diluted 20%.

Over 50 years ago, Kracke and Teasley (9) recognized the importance of diluting blood in cultures to neutralize its normal bactericidal properties; these researchers found, on the basis of studies of experimentally induced bacteremias in rabbits, that 1:15 to 1:20 dilutions are optimal for the recovery of bacteria from blood cultures. A 1:10 to 1:20 blood/medium ratio was recommended by Bartlett et al. (3) and was used by most respondents to two surveys of blood culture practices in clinical laboratories in 1971 and 1981, respectively (2, 5). Since most respondents to these surveys also used blood culture media containing 0.025 to 0.05% sodium polyanetholsulfonate (SPS), and since Lowrance and Traub (11) demonstrated that 0.025% SPS abolished the bactericidal activity of 20 and 50% fresh sera against small inocula of bacteria, it seemed reasonable to determine whether Kracke and Teasley's original recommendations, based on cultures in media without SPS, could be modified by reducing the currently recommended ratio of blood to medium.

Because of the direct, documented relationship between the volume of blood cultured and the yield of microorganisms (12, 14; J. H. Tenney, L. B. Reller, C. W. Stratton, and W.-L. L. Wang, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 16th, Chicago, Ill., abstr. no. 309, 1976), the advantages of adding a larger volume of blood to a relatively smaller volume of broth in a relatively smaller bottle are considerable. In a parallel evaluation of over 5,000 blood cultures, Reller et al. (L. B. Reller, K. A. Lichtenstein, S. Mirrett, and W.-L. L. Wang, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, C177, p. 306) found no significant difference in the rates of recovery of all organisms from cultures of 10 ml of blood diluted 1:5 or 1:10; however, positive results were detected sooner ($P < 0.01$) in the blood cultures diluted 1:5. For specimens from patients receiving effective antistaphylococcal therapy, *Staphylococcus aureus* was detected more frequently ($P < 0.01$) in blood cultures diluted 1:10. In a study of only 2,550 cultures, Salventi et al. (12) failed to demonstrate significant enhancement or inhibition of organism recovery for cultures in which the blood/medium ratio was 1:4 to 1:30; however, the number of positive cultures was too small for adequate statistical analysis of the variable, and the effects of effective antimicrobial therapy on the recovery of bacteria from blood were not specifically examined.

The purpose of this study was to examine the relative yields of organisms from equal volumes of blood diluted 1:10 (10%, vol/vol) and 1:5 (20%, vol/vol) in broth and to determine what effects, if any, the presence of antimicrobial agents in the blood at the time of specimen collection had on the organism yield of the cultures.

MATERIALS AND METHODS

Blood cultures were collected by members of a phlebotomy team from patients with suspected septicemia. At least two sets of blood cultures were collect-

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ed from each patient during each suspected septic episode (14). Each patient was classified according to record of antimicrobial therapy and whether the isolates from his or her blood were susceptible or resistant to the antibiotic concentration predicted, on the basis of dosage, to have been present in his or her serum at collection. Group 1 comprised patients who had received no antimicrobial therapy 7 days before blood was taken. Group 2 comprised patients whose blood culture isolates were resistant to the antimicrobial agents received before blood was taken and patients who had not received β-lactam antibiotics, aminoglycosides, or bacteriostatic agents 24 h, 4 days, or 24 h, respectively, before blood was taken. Group 3 comprised patients who had received, before blood was taken, antimicrobial agents in doses resulting in concentrations anticipated to exceed those active in vitro against the organisms isolated from their blood.

From each patient, 30 ml of blood was collected per blood culture set (14); 10 ml was inoculated into each of two 100-ml bottles containing tryptic soy broth with SPS under vacuum in an atmosphere containing CO_2 (Difco Laboratories) and into one 50-ml bottle containing Trypticase soy broth with SPS under vacuum in an atmosphere containing CO_2 (BBL Microbiology Systems). (Tryptic and Trypticase soy broths were presumed to be equivalent since no significant differences between them had been noted in a parallel clinical study of 2,632 blood cultures [7].) One of the 100-ml bottles was transiently vented before incubation, and the other was not. An aerobic venting unit was permanently applied to the 50-ml BBL bottle before incubation at 35°C in air. Each bottle was examined for visible evidence of growth several hours after inoculation, daily for 7 days, and after 14 days of incubation. In addition, a portion from each bottle was subcultured onto chocolate blood agar (incubated in 10% CO_2 for 48 h) 6 to 12 and 48 h after incubation (13).

Because of the mutually exclusive atmospheric requirements or preferences of certain groups of bacteria, statistical analysis of results was limited to the two vented blood culture bottles containing 10 ml of blood each. The results were analyzed statistically as described by Ilstrup (8).

Additional studies were made of the chemical compositions, pH values, and CO_2 and O_2 partial pressure values of the media in the Difco and BBL bottles before and after the addition of 10 ml of fresh human volunteer blood to each bottle. Sequential 0.3-ml portions were removed from each bottle for blood gas analysis (178 pH/Blood Gas Analyzer; Corning Glass Works) after the bottle contents had been thoroughly mixed.

RESULTS

There were 6,108 pairs of vented blood culture bottles that we could evaluate; 410 (6.7%), representing 269 patients, yielded growth in one or both bottles. Of the 269 patients represented by these pairs, 38 (14%) had polymicrobial bacteremia. Of the 6,108 culture sets, 94 (2.5%) yielded presumed contaminants (i.e., *Staphylococcus epidermidis*, corynebacteria, and *Bacillus* spp.). Statistically significant differences (classified by genus or species) between rates and times of isolation for blood diluted 10% and those for blood diluted 20% are shown in Table 1. For all groups collectively ($P < 0.05$) and group 3 ($P < 0.01$), *Enterobacteriaceae* and *Pseudomonas* spp. were isolated significantly more often in blood cultures diluted 10%. For all groups collectively, blood cultures diluted 10% were also positive earlier ($P < 0.01$). The eight group 3 cultures positive only with blood diluted 10% led to the discovery of previously unrecognized foci of infection in three patients and to an alteration in therapy for four patients. The 32 cultures yielding *Pseudomonas* spp. plus *Enterobacteriaceae* (of which 16 were *Escherichia coli*) only with blood diluted 10% represented 15 patients whose bacteremias were not detected in any other blood cultures and another 15 patients whose bacteremias were detected in other blood cultures diluted 10 and 20% or only in other blood cultures diluted 20%.

Fourteen cultures representing nine patients yielded *Staphylococcus aureus* only in blood diluted 10% (Table 1). Previous cultures of specimens from three of these nine patients were positive with blood diluted 10 and 20%. For three patients, the positive cultures were of uncertain clinical significance and prompted no change in patient management. For two patients, the positive cultures confirmed a clinical impression of staphylococcal infection. For one patient, the isolation prompted a change in therapy since staphylococcal infection had not been suspected. Seven cultures representing six patients yielded group D streptococci only with blood diluted 10%: three patients had had previous positive blood cultures; one had not been suspected of having an infection; for another, the positive culture was of doubtful clinical significance; for the third, the positive culture confirmed a suspected enterococcal infection. Twelve cultures representing seven patients yielded *Candida* spp.: four patients had had other positive blood cultures, and for three patients, the isolates were of doubtful clinical significance.

The only organism isolated significantly sooner and more frequently from blood diluted 20% was *Streptococcus pneumoniae* (Table 1). Of the 10 cultures yielding pneumococci with blood diluted 20%, 5 represented only three patients: two had pneumonia and one had acute maxillary sinusitis of previously undocumented etiology. The isolation of pneumococci in the cultures representing these three patients led to a change in therapy from broad-spectrum antibiotics, including aminoglycosides, to specific therapy with penicillin. The other five cultures yielding pneumococci with blood diluted 20% represented five patients whose pneumococcal bactere-

TABLE 1. Differences in yields between blood cultures diluted 10 and 20%

Organism(s)	Patient group	No. of cultures positive with indicated dilution			Faster culture	Avg difference in culture time (days)	*P* value
		10%	20%	Both			
Enterobacteriaceae	All	27[a]	13	73	10%	0.8	<0.01
	1	20[a]	9	54	10%	0.9	
Pseudomonas	All	5	2	21			<0.01
Enterobacteriaceae + *Pseudomonas* spp.	All	32[a]	15	94	10%	0.8	<0.01
	3	8	0	14			
Staphylococcus aureus	All	14[b]	2	38	10%	1.1	<0.05
	1	8	2	35	10%	1.2	<0.05
Streptococcus spp.							
S. pneumoniae	All	1	10[c]	7	20%	0.7	
	1	1	10[c]	7	20%	0.7	
Group D	All	7[a]	0	15			
Candida spp.	All	12[a]	3	6			

[a] Blood cultures diluted 10% were positive significantly more often ($P < 0.05$) than were cultures diluted 20%.
[b] Blood cultures diluted 10% were positive significantly more often ($P < 0.01$) than were cultures diluted 20%.
[c] Blood cultures diluted 20% were positive significantly more often ($P < 0.05$) than were cultures diluted 10%.

mias were documented in other sets of blood cultures, including those diluted 10%.

Differences in the chemical compositions of the broths in Difco and BBL blood culture bottles were limited to the concentrations of calcium and magnesium, which were, respectively, 2.6 and 2.6 mg/dl in BBL Trypticase soy broth and 0.3 and 0.76 mg/dl in Difco tryptic soy broth. The pH and O_2 and CO_2 partial pressure determinations over time are shown in Fig. 1.

DISCUSSION

There were several statistically significant differences in isolation rates observed between blood cultures diluted 10% and those diluted 20%. Significantly more cultures yielded *Staphylococcus aureus* (all patient groups), group D streptococci (all patient groups), *Candida* spp. (all patient groups), and *Enterobacteriaceae* plus *Pseudomonas* (all patient groups) when the blood was diluted 10%. Conversely, *Streptococcus pneumoniae* was isolated significantly more frequently from blood cultures diluted 20%. From all patients receiving presumably effective antimicrobial therapy (group 3) there were (excluding presumed contaminants) 19 cultures positive with blood diluted 10 and 20%, 18 positive only with blood diluted 10%, and none positive only with blood diluted 20% ($P < 0.01$). Although there were no statistically significant differences in rates of isolation of staphylococci, streptococci, and gram-negative bacilli between vented, vacuum blood culture bottles containing tryptic soy broth (Difco) and vented, vacuum blood culture bottles containing Trypticase soy broth (BBL) in a previous parallel clinical study at this laboratory (7), *Streptococcus pneumoniae* was not isolated during that study from any cultures in either type of bottle. The possibility that the difference in pneumococcus isolation was medium related led to the chemical analysis of the medium in each type of bottle and the finding that the calcium and magnesium contents were higher in Trypticase soy broth than in tryptic soy broth. The pH of tryptic soy broth was lower before and after the addition of fresh human blood than that of Trypticase soy broth. Blood gas concentrations and pH changes were very similar to those observed by Beaman et al. (4) after the addition of blood to Difco tryptic soy broth blood culture bottles. Although the optimal pH for the growth of pneumococci is 7.4 to 7.8 (1), Lopez et al. (10) demonstrated comparable growth rates of pneumococci in media with initial pH values of 8.0 and 6.6; therefore, it seems unlikely that the difference in pH values between tryptic soy broth and Trypticase soy broth observed in our study sufficiently ac-

counts for the difference in pneumococcus recovery. Divalent cations, however, have been found to stabilize another species that readily undergoes autolysis, *Neisseria gonorrhoeae* (6), and might account for the difference in pneumococcus recovery. Another possible explanation for the greater recovery of pneumococci from blood cultures diluted 20% is that the survival of pneumococci is enhanced by the presence of a catalase such as that provided by erythrocytes (1), which were obviously present in a higher proportion in the blood cultures diluted 20% than in those diluted 10%. Further studies are needed to determine the relative importance of these variables.

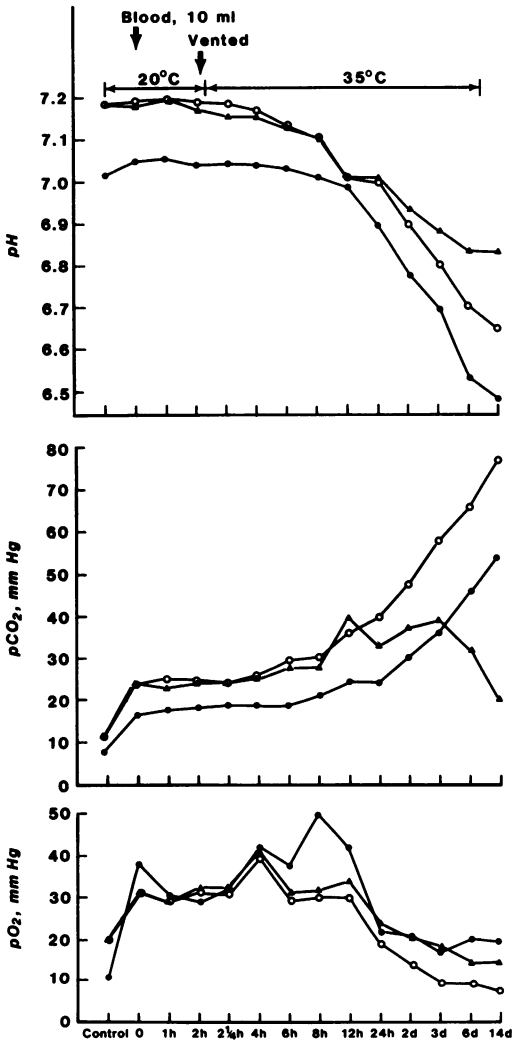

FIG. 1. Measurements of pH, partial pressure of O_2 (pO_2), and partial pressure of CO_2 (pCO_2) in transiently vented vacuum blood culture bottles (50 ml) containing Trypticase soy broth (○), in permanently vented vacuum blood culture bottles (50 ml) containing Trypticase soy broth (▲), and in transiently vented vacuum blood culture bottles (100 ml) containing tryptic soy broth (●) before and after the addition of 10 ml of fresh human volunteer blood.

Regardless of the reasons for the significantly greater recovery of pneumococci from blood cultures diluted 20%, blood cultures diluted 10% were positive for gram-negative bacilli, group D streptococci, *Staphylococcus aureus*, and *Candida* spp. in significantly greater numbers, apparently owing to the greater dilution of blood and the resulting reduction of the normal bactericidal properties or components of blood. Also of importance was the dilution of antimicrobial agents which, given the dosage schedules and in vitro susceptibility tests, should have been effective against the bacteria isolated.

In conclusion, the results of this study do not support an unqualified recommendation for a 1:5 (20%) dilution of blood in broth containing SPS. Our study was limited to a comparison of vented vacuum blood culture bottles; the results cannot be applied to unvented bottles. Also, certain organisms (e.g., *Haemophilus influenzae, Neisseria meningitidis*) were either not encountered or encountered too infrequently for statistical analysis. Because we obtained at least two separate sets of blood cultures from each patient per septic episode, in only a few instances did blood cultures diluted 10% represent the only means of isolation and thus affect clinical management. However, growth was detected significantly sooner in blood cultures diluted 10% than in those diluted 20%. Although the results of blood cultures diluted 20% might be acceptable when at least two separate sets of blood cultures per septic episode are used, the results of our study also demonstrate that, with the possible exception of *Streptococcus pneumoniae*, blood cultures diluted 10% provided the greatest and fastest yield of microorganisms most frequently associated with septicemia in adults.

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